**FIGURE 1A** 

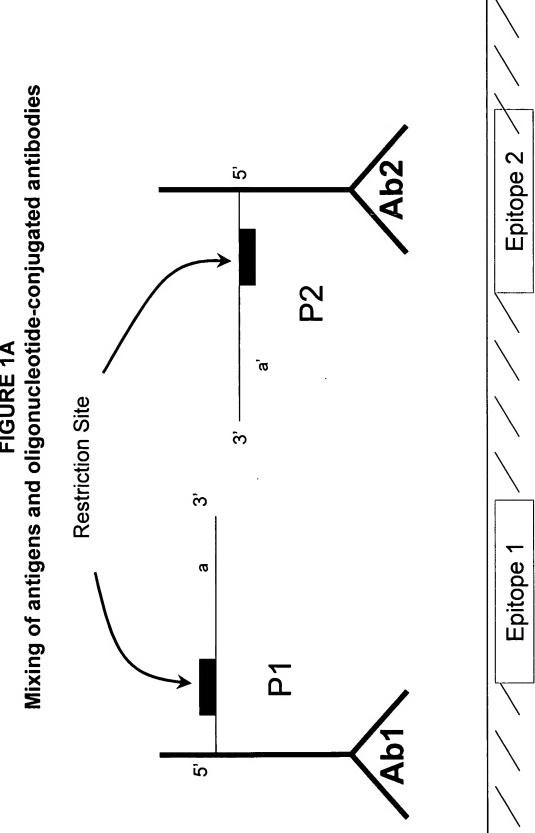
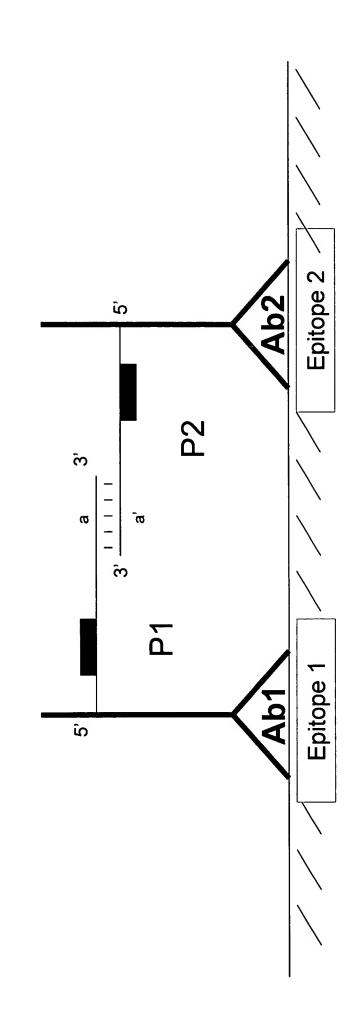
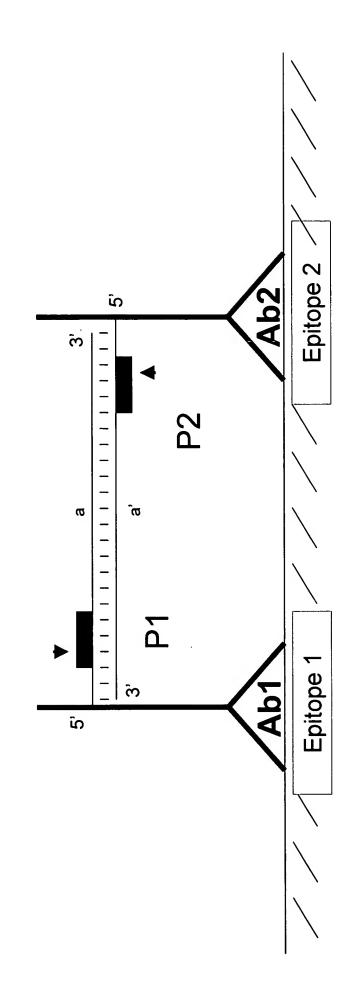


FIGURE 1B
Hybridization of adjacent oligonucleotide probes

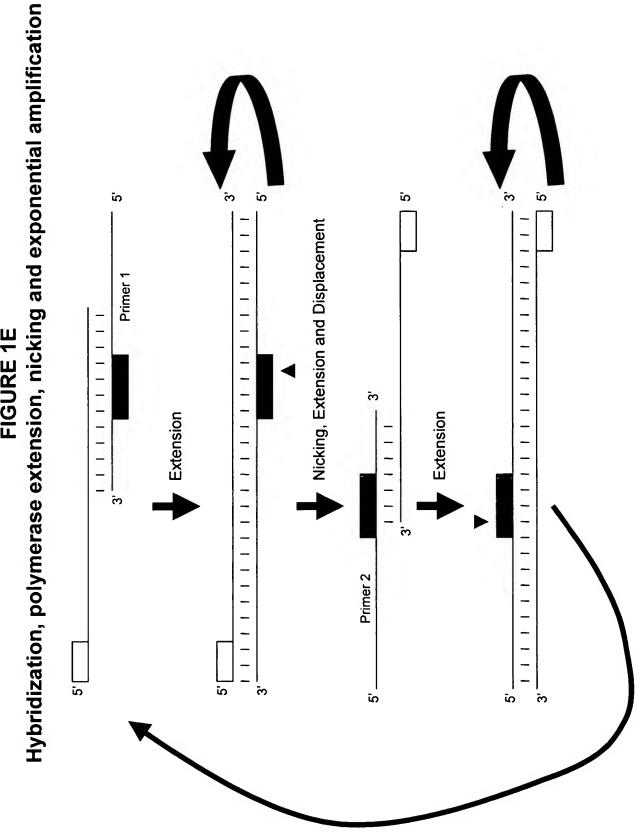


Polymerase extension and restriction enzyme nicking **FIGURE 1C** 



Extension, displacement and linear amplification Epitope 2 Ω က် ດິ 2 က ري کا ດິຼ က် FIGURE 1D ์ซ  $\boldsymbol{\sigma}$ , , **№**5'<sub>1</sub> m  $\hat{\omega}$ 75/4 જ 5 Epitope 1 က် Ab1 ũ Restriction Site Partial

FIGURE 1E



Mixing of antigens and oligonucleotide-conjugated antibodies FIGURE 1F

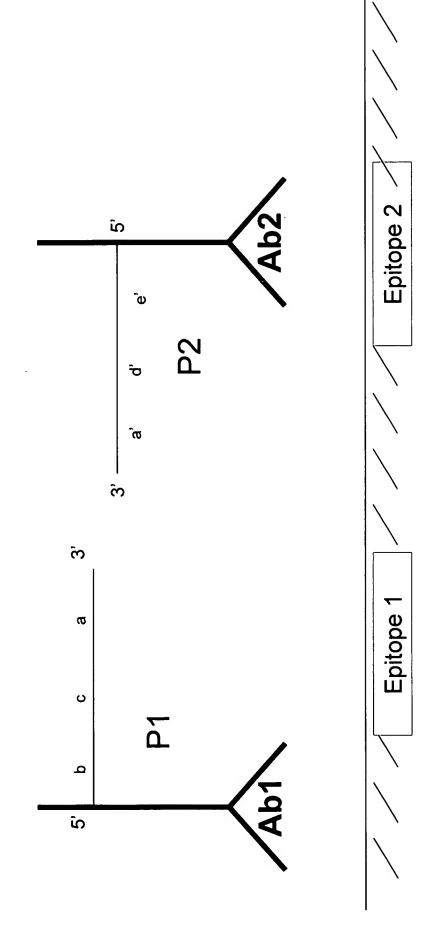


FIGURE 1G
Hybridization of adjacent oligonucleotide probes

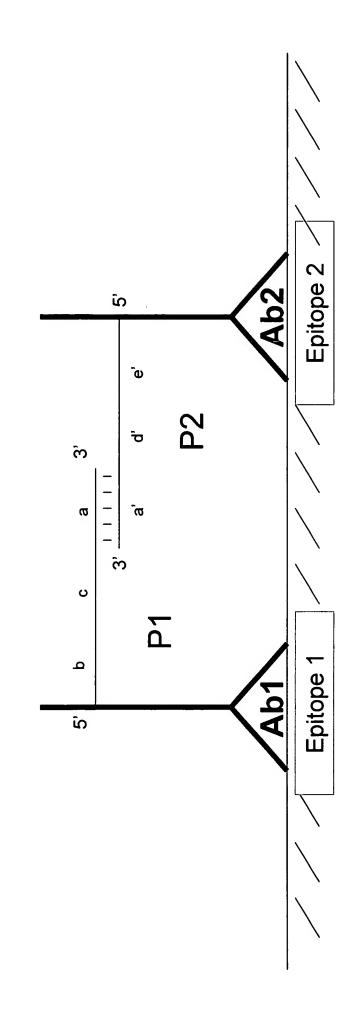
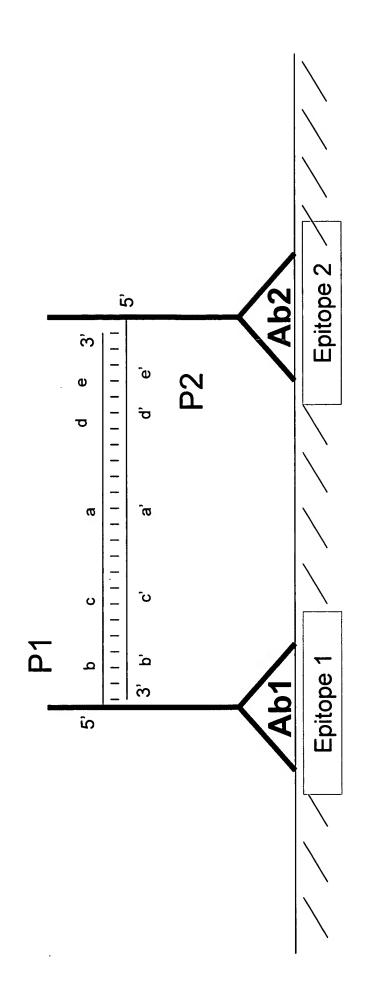
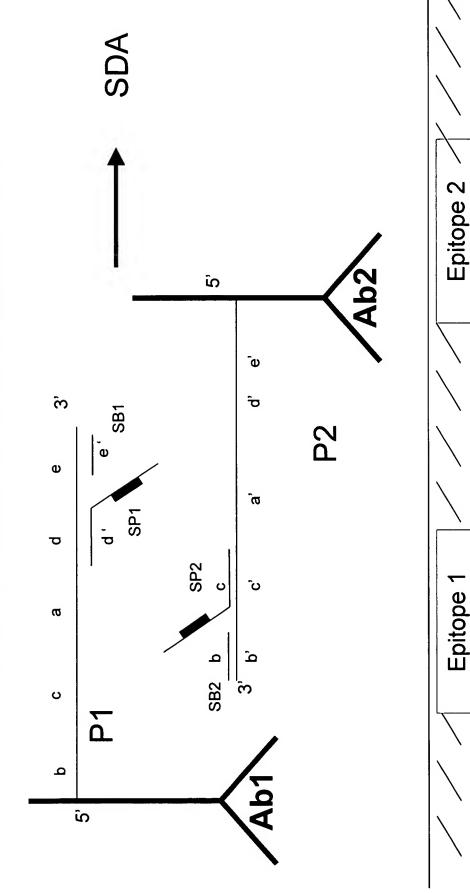


FIGURE 1H
Extend oligonucleotide probes with polymerase



Denature probe-extension duplex and bind SDA primers (SP1, SP2) and bumpers (SB1,SB2) **FIGURE 1I** 



**FIGURE 1J** 

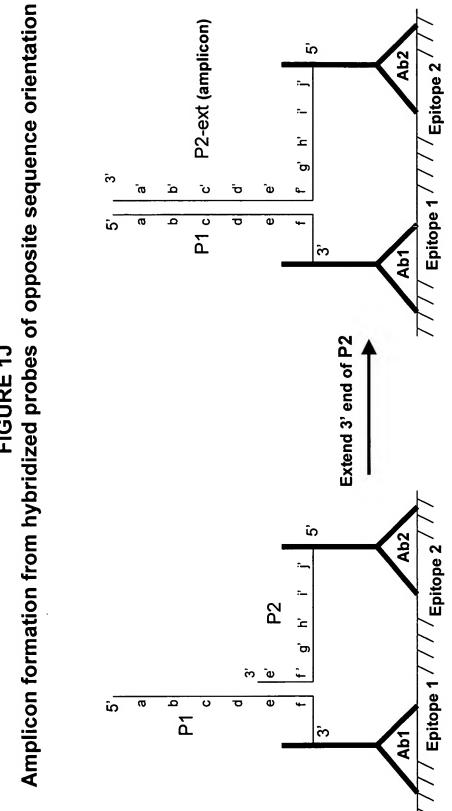


FIGURE 2A
Hybridization of splint oligonucleotide

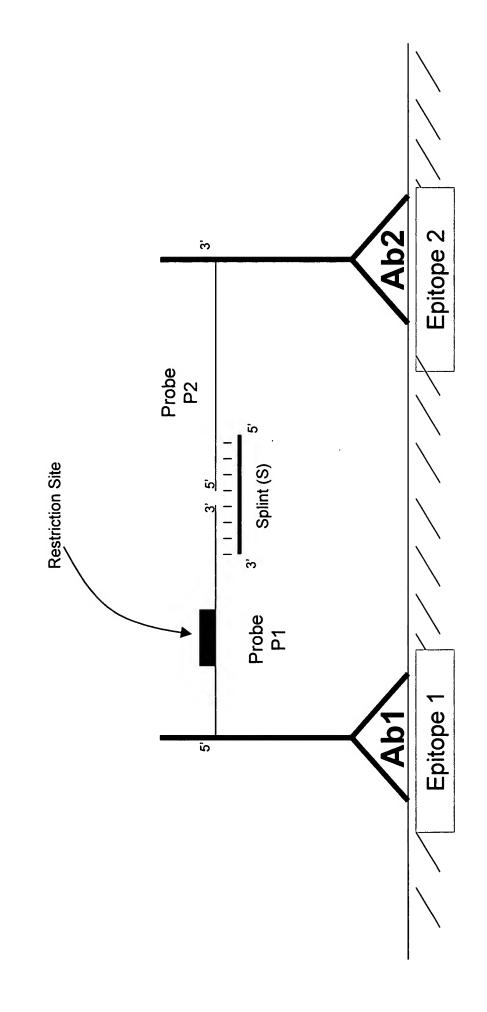


FIGURE 2B Ligation of adjacent oligonucleotide probes

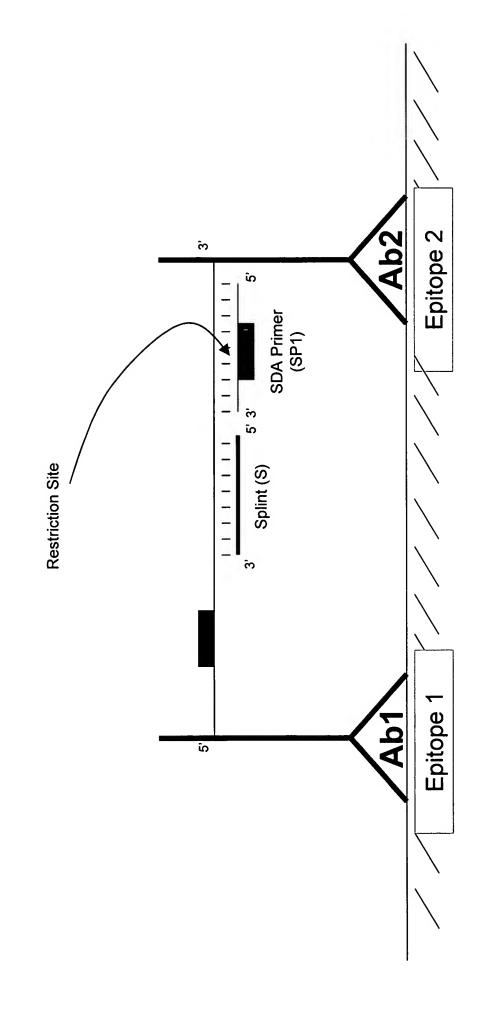


FIGURE 2C

DNA polymerase extension and displacement

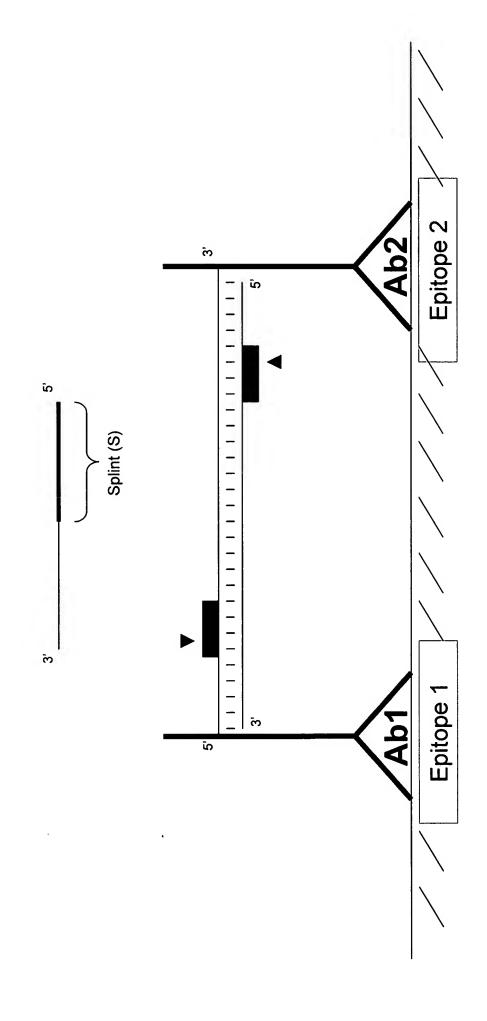
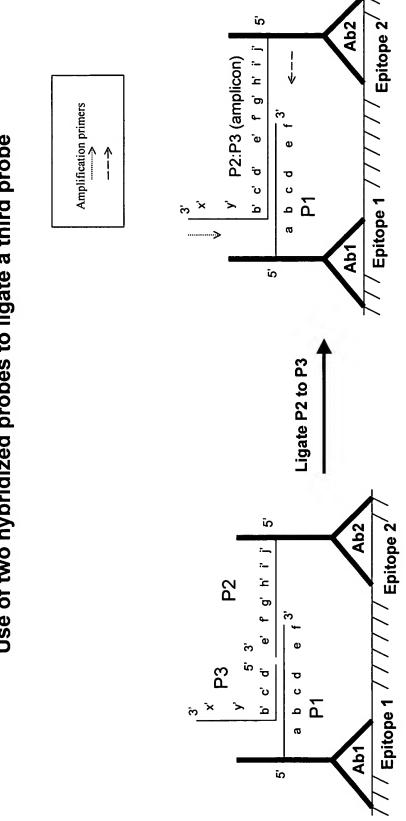


FIGURE 2D
Use of two hybridized probes to ligate a third probe



P2:P3 (amplifiable) Amplification primers Ω È Use of two hybridized probes in opposite sequence ້ တ ۵. ٠ σ œ σ P1 c Ω Ф þ a orientation to ligate a third probe က် **FIGURE 2E** Ligate P2 to P3 3' P2 (not amplifiable) Ŝ P3 <u>`</u>\_ **ົ** ຕ م` ์ต Ω ပ က် ລີ Ø Ω ပ Φ 7 ŝ

Epitope 2

Epitope 1 /

Epitope 2

Epitope 1 /

Ab1

Ab1

Epitope 2 Ab2 Ω Sequence slh-2 Primer က် Probe - P2 က က Detector Region Sequence srh-1 Probe- P1 Primer **Tether Oligonucleotide** Restriction Site Ω Ŝ ဥ Epitope 1 က

FIGURE 3A Single-tether oligonucleotide

Epitope 2 Ab2 ΩĨ က် Single-tether oligonucleotide: extension and displacement Primer slh-2 Detector Region Primer srh-1 Restriction Site ີ່ດ က် Epitope 1 က် A<sub>b</sub>1 ດົ

**FIGURE 3B** 

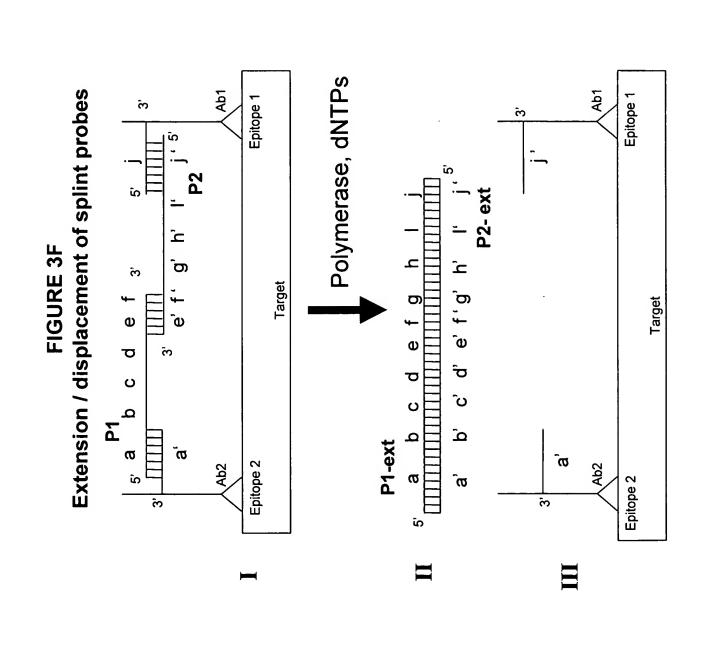
SDA Primer SP2 ŝ  $\mathbf{\omega}$ Nicking, extension, displacement and capture Polymerase Primer 2 Nick, Extend, Displace ŝ Nick and Displace ŝ ŝ Detector Region Primer 2 Sequence Primer 1 Sequence Restriction Site û

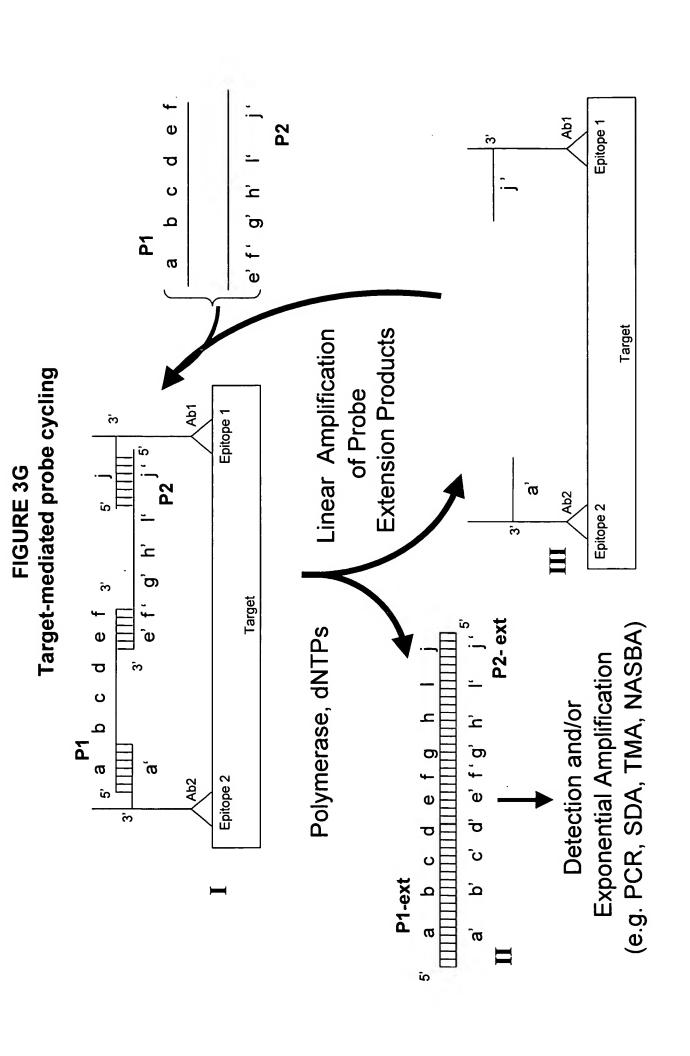
**FIGURE 3C** 

Nick, Extend, Displace Polymerase Nicking, extension, displacement and capture (cont.) Amplification Exponential G щ SDA Primer, SP1 **FIGURE 3D** Primer 1 Ш

| Epitope 2 က Ω ري ا <u>.</u> \_ ნ က် Φ က Q ပ Ω ũ 7 ື້ ത Epitope 1 Ω က်

FIGURE 3E Splint probes (3'/3' configuration)

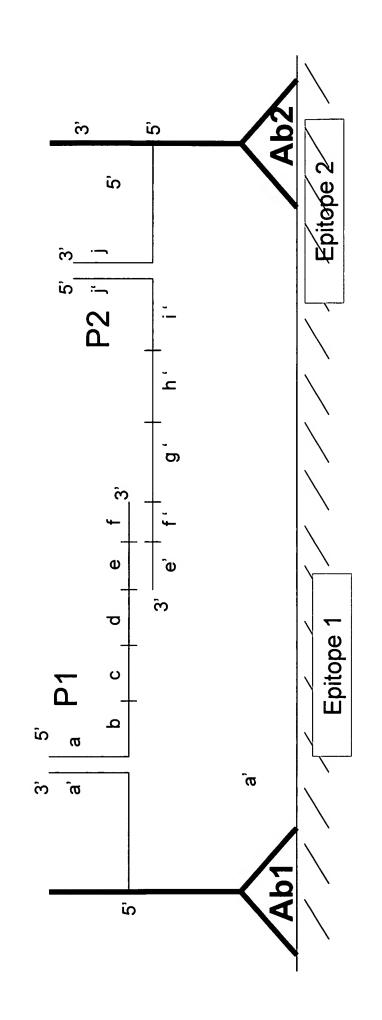




က် Epitope 2 ົດ Splint probes (5'/3' configuration) <u>,</u> ರಾ က Φ က် O Epitope 1 ပ Ω Ω σ ື້ က် ື ຕ 5,

FIGURE 3H Splint probes (5'/3' configuration)

FIGURE 3I Splint probes (5'/5' configuration)



| Epitope 2 Ω က **P**2 ŝ Ñ Splint probes (3'/3' configuration) . ح Ligation splint , თ D Ω <del>က</del> သိ Φ œ<sup>¯</sup> က် ס ပ Ω ũ 7 **ື** ຫ σ Epitope 1 Ωí က်

FIGURE 3J

Epitope 2 ũ က splint ~ က် ດິ Splint probes (3'/3' configuration) **P**2 ũ . ත **FIGURE 3K** ჯ უ Φ ŝ ס ပ Ω Ω 7 ์ซ  $\boldsymbol{\omega}$ Epitope 1 ĝ Ab1 က

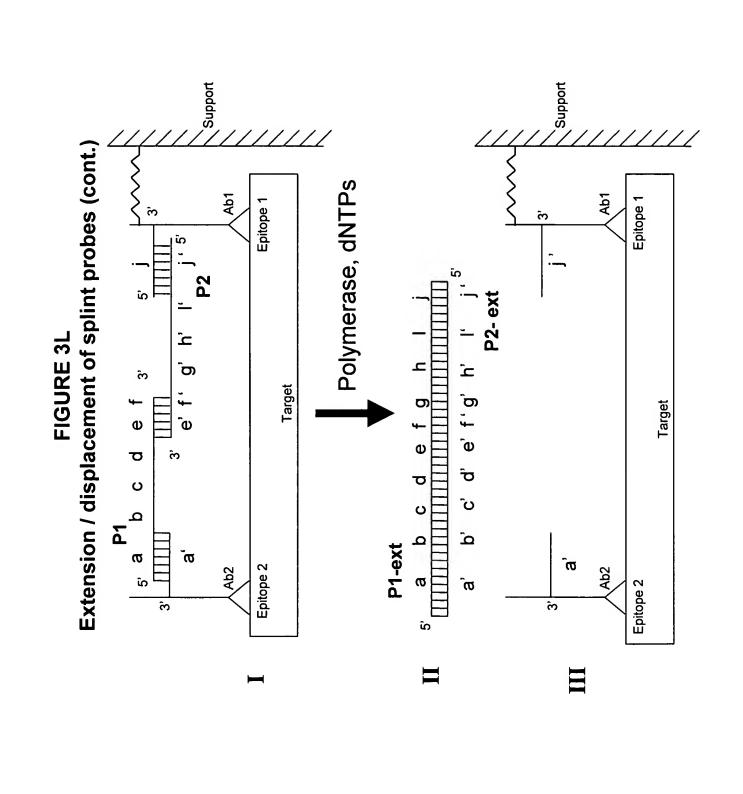
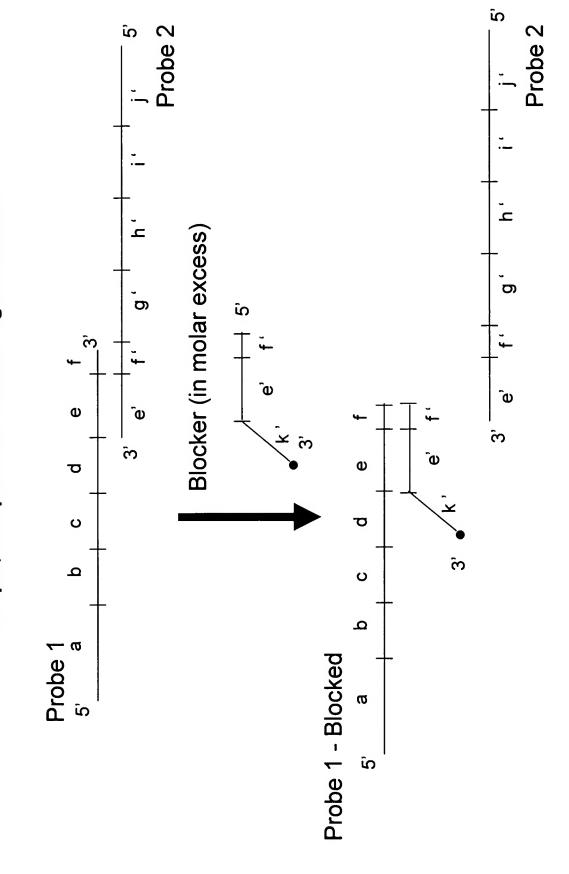


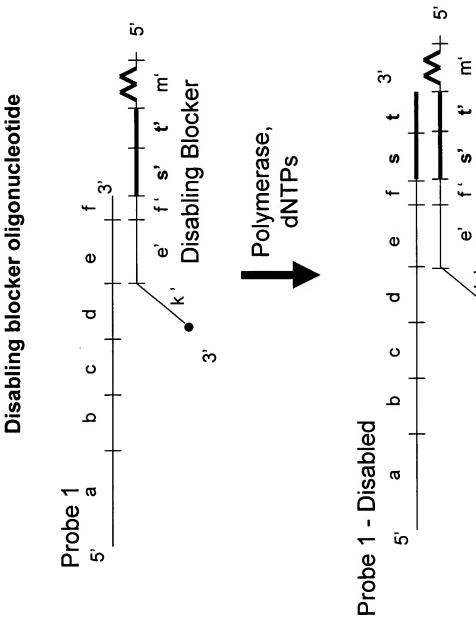
FIGURE 4A Simple, competitive blocker oligonucleotide



ũ Probe 2 Probe 2 Recessed, competitive blocker oligonucleotide Blocker (in molar excess) D D œ<sup>ˆ</sup> **FIGURE 4B** Φ က် ŝ Φ σ œ က ပ  $\boldsymbol{\sigma}$ ٩ ပ က် Ω Probe 1 Probe 1 - Blocked ത کر ک ũ

Ω

FIGURE 4C
Disabling blocker oligonucleotide



က်

FIGURE 4D
Displaceable blocker oligonucleotide

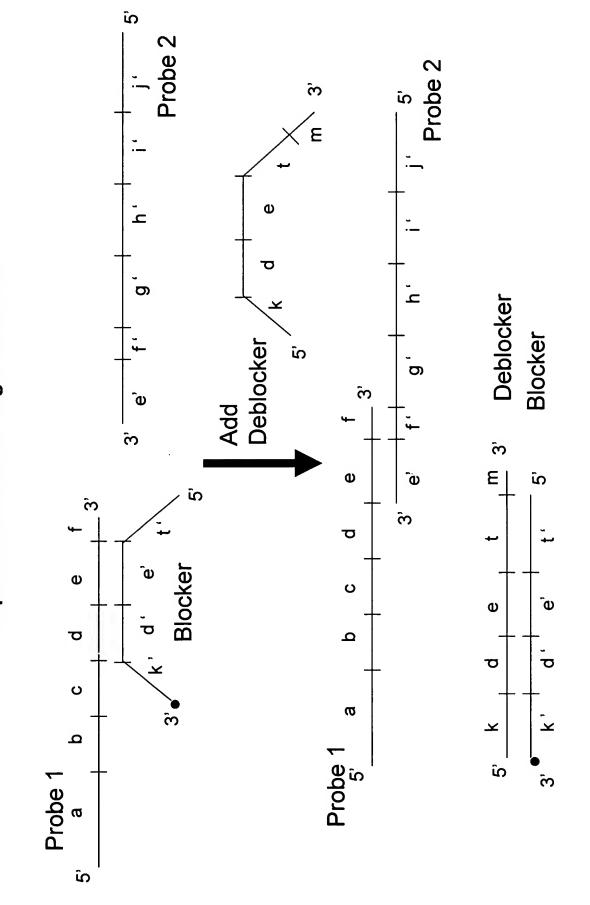
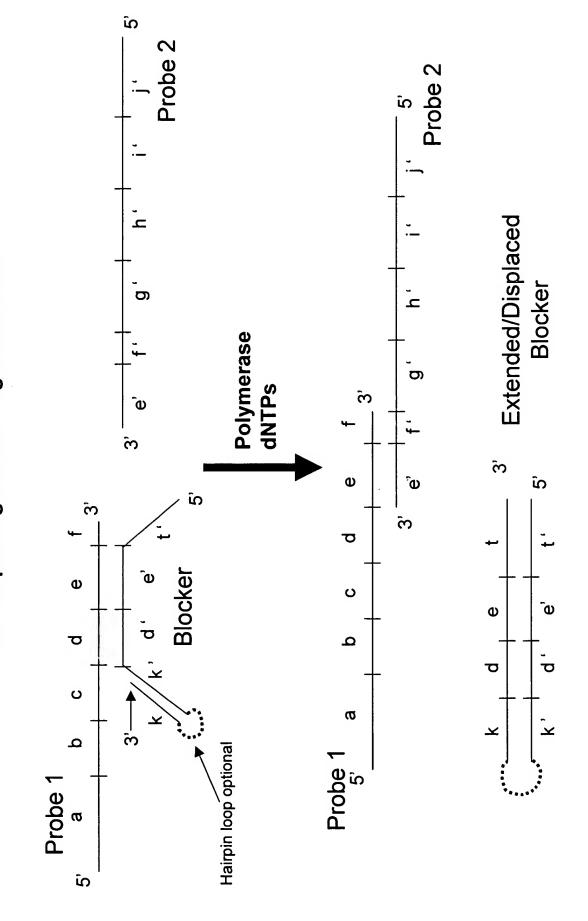
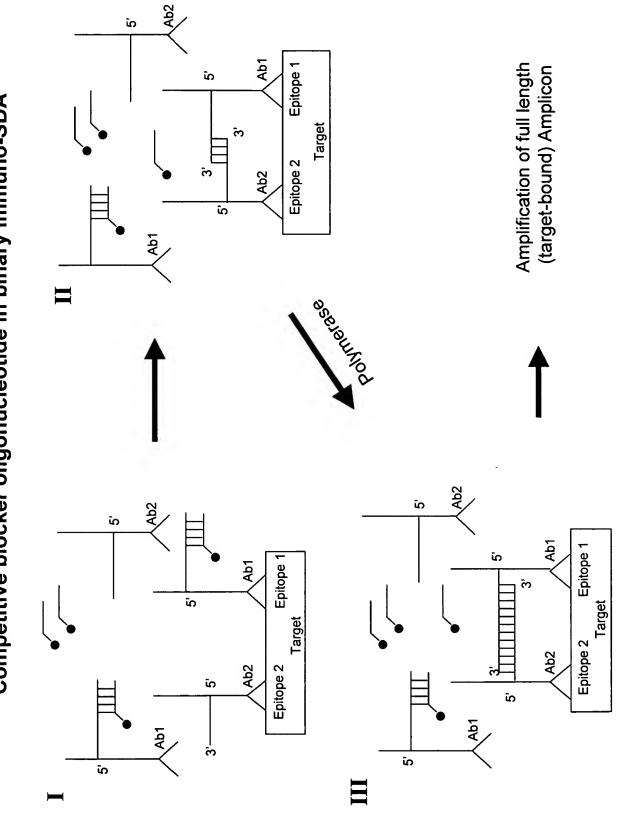


FIGURE 4E Self-displacing blocker oligonucleotide



ũ Probe 2 ũ Probe 2 ည် Use of 3' probe tail to stabilize probe-blocker duplex Ň ຸນ Ň က် က D D **FIGURE 4EE** × œ Ū Φ œ<sup>^</sup> **Blocker** က် က် Œ Φ σ ပ  $\boldsymbol{\sigma}$ Ω ပ ۵ Probe 1 Probe 1 - Blocked ത Ω Ω

Competitive blocker oligonucleotide in binary immuno-SDA **FIGURE 4F** 



Disabling blocker oligonucleotide in binary immuno-SDA **FIGURE 4G** 

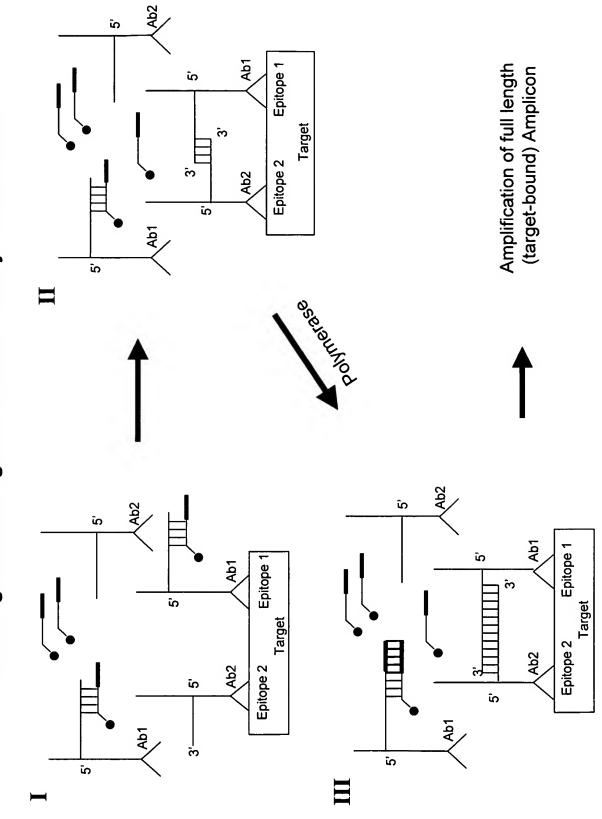
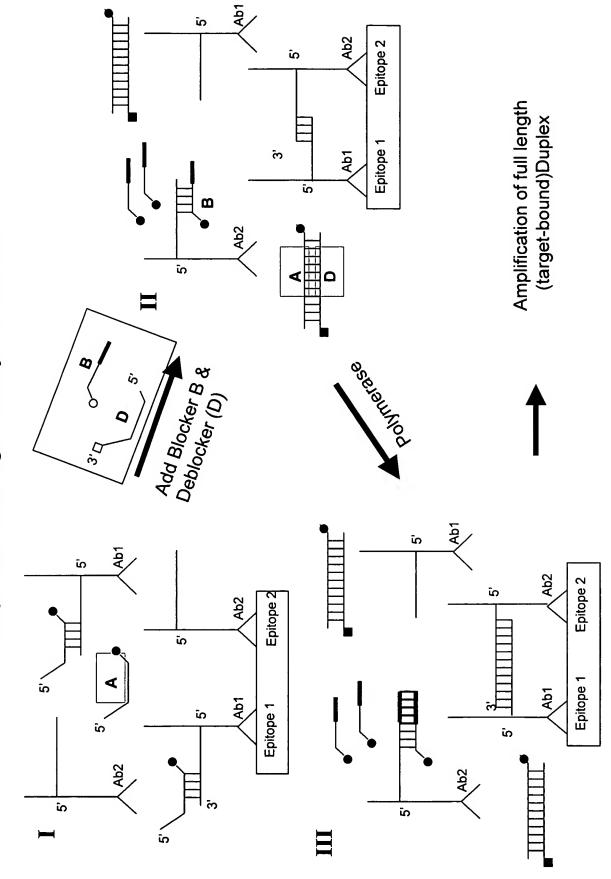
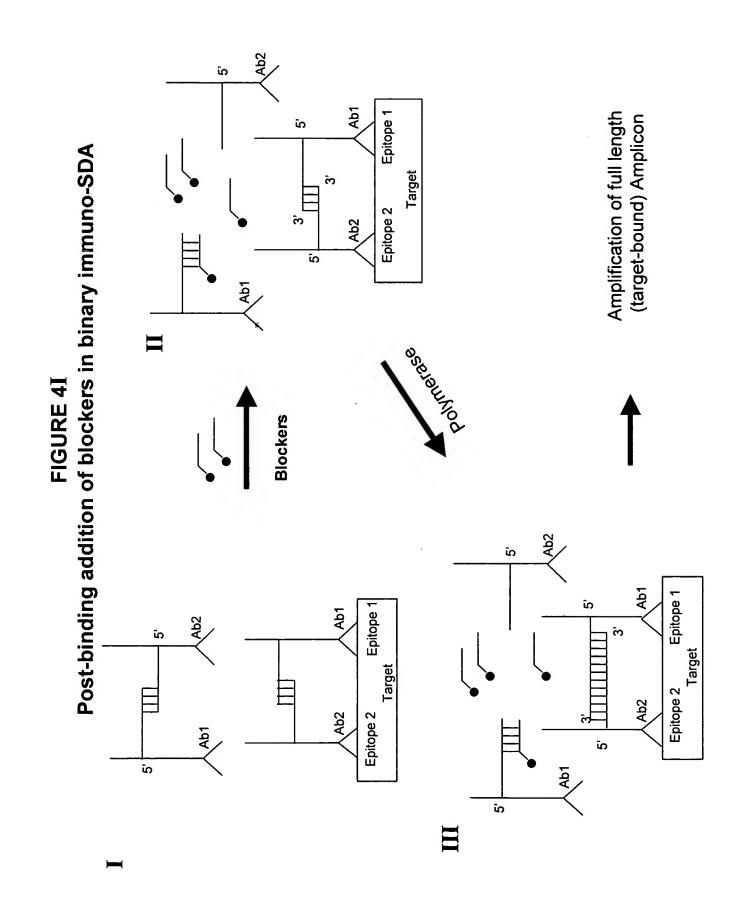


FIGURE 4H
Step-wise blocking in binary immuno-SDA





Epitope 2 Ab2 ĝ Probe (P2) Sequence 1 Primer က် . რ Detection Region Splint (S) Sequence 2 Primer RNA Polymerase Promoter ũ Probe (P1) ດ໌ Epitope 1 Ab1 Ś

FIGURE 5A Splint oligonucleotide hybridization

Epitope 2 Ab2 Ω က် Sequence 1 Primer Detection Region Primer Sequence 2 RNA Polymerase Promoter ີດ က် Epitope 1 က A<sub>b</sub>1 ດ໌

FIGURE 5B Extension and displacement

RNA polymerase activity, hybridization and extension **FIGURE 5C** 

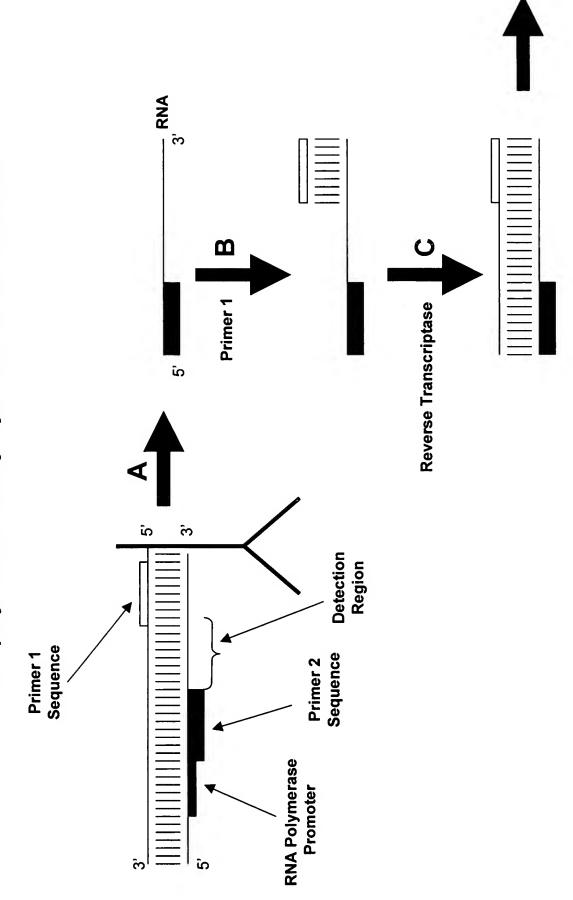


FIGURE 5D RNase H activity, hybridization and extension

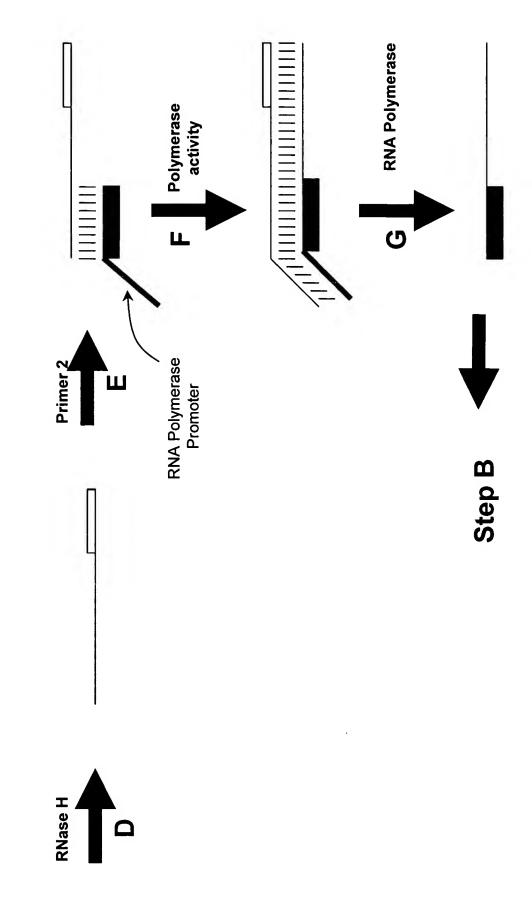
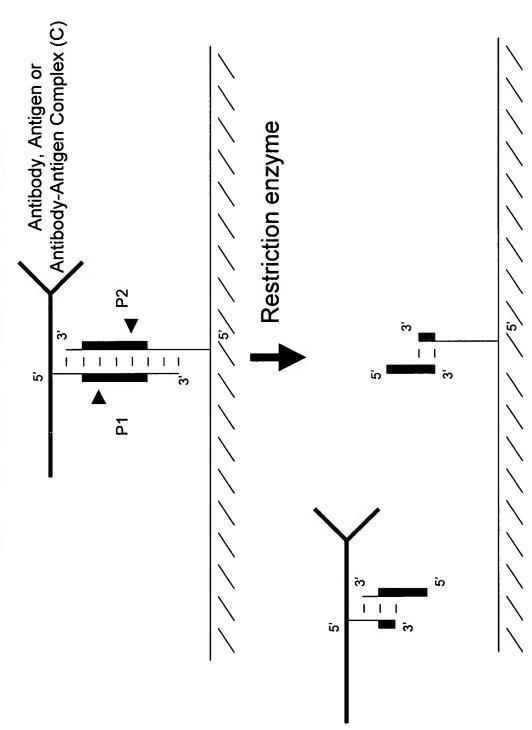


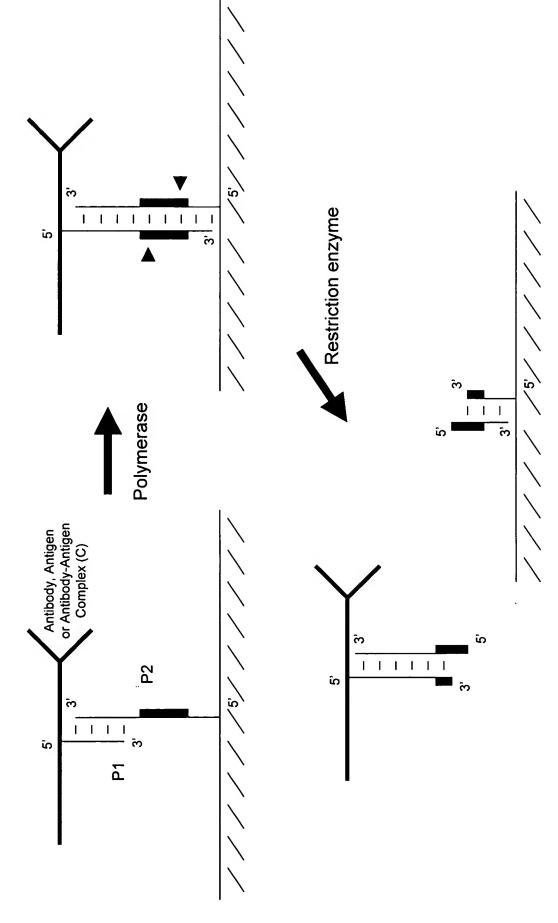
FIGURE 6A Restriction endonuclease-mediated release



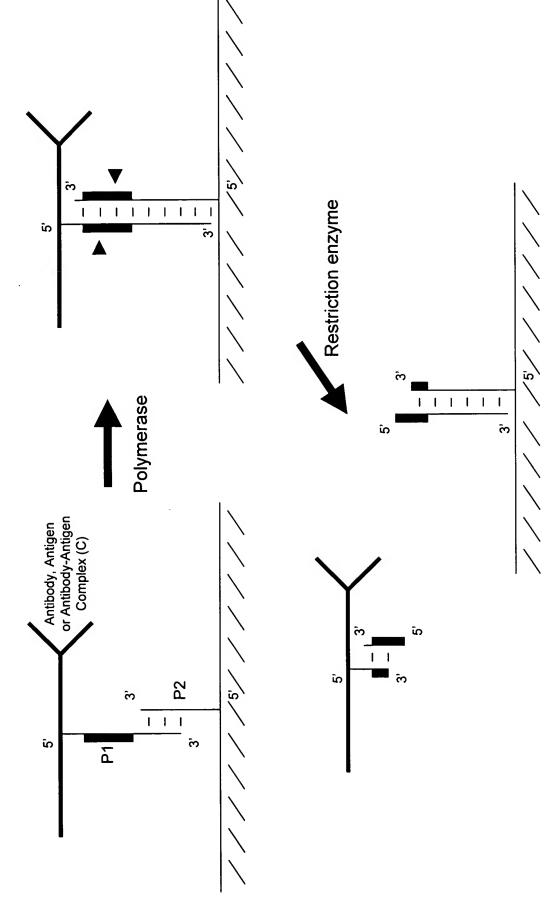
1 1 ັດ ۵. م. က Displacement oligonucleotide Restriction endonuclease-mediated release ດົ က် Antibody, Antigen or Antibody-Antigen Complex (C) Restriction FIGURE 6B enzyme က် ڝٙ P2 ŝ  $1 \quad 1 \quad 1 \quad 1$ ົດ ດົ **A**°.

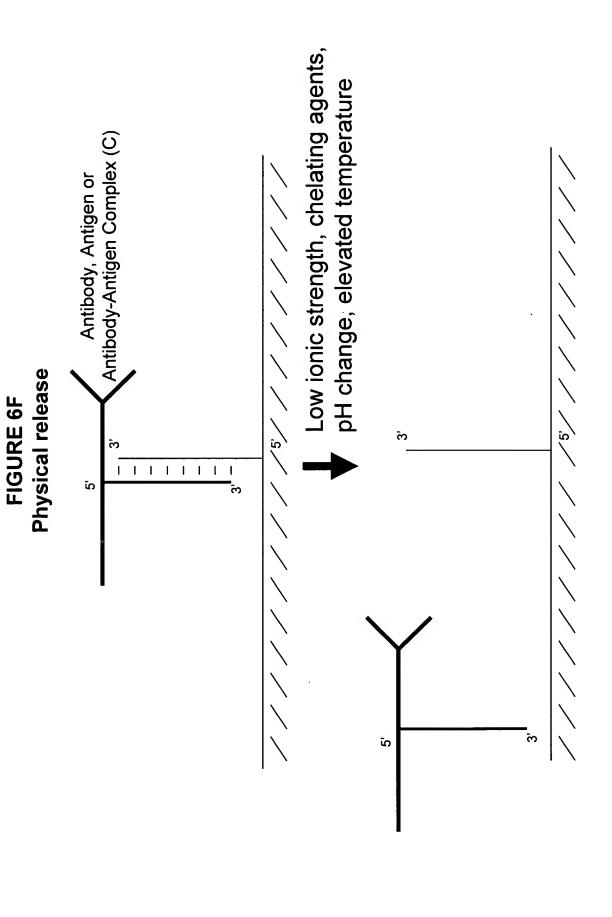
Antibody, Antigen or Antibody-Antigen Complex (C) Restriction enzyme Restriction endonuclease-mediated release **FIGURE 6C** <u>1 | ا ا</u> က် ີດ ₽ **▼** က

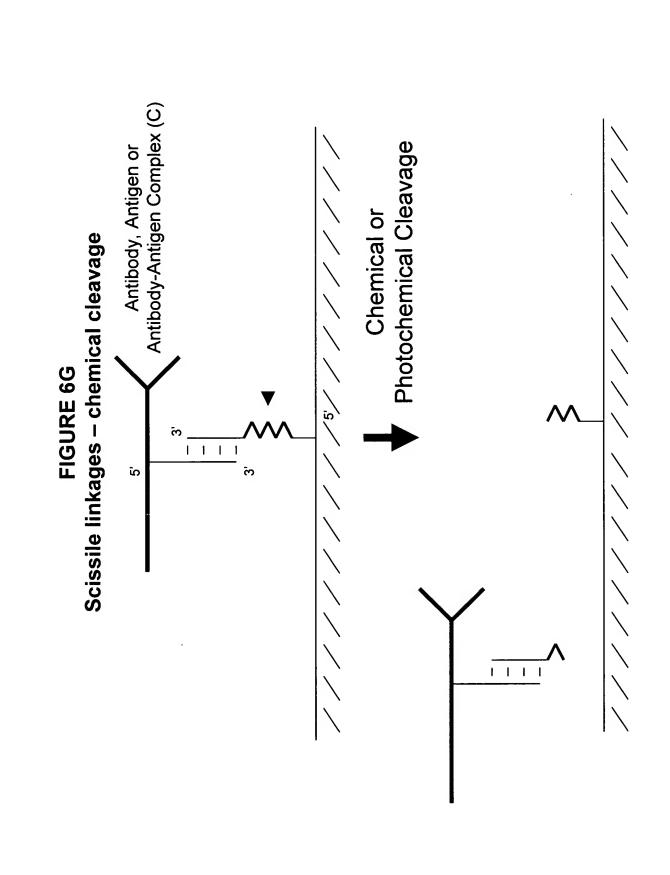
Polymerase and restriction endonuclease-mediated release **FIGURE 6D** 

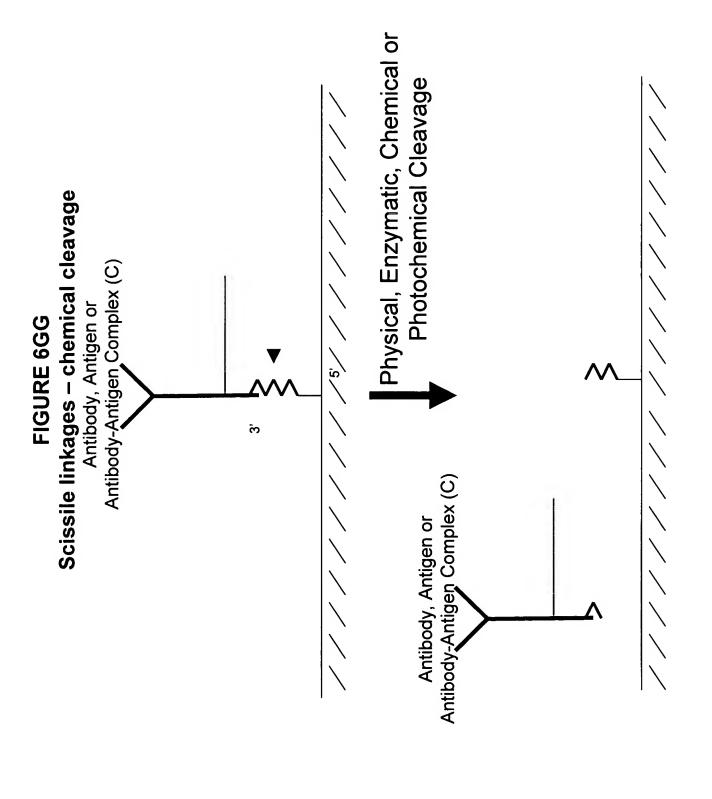


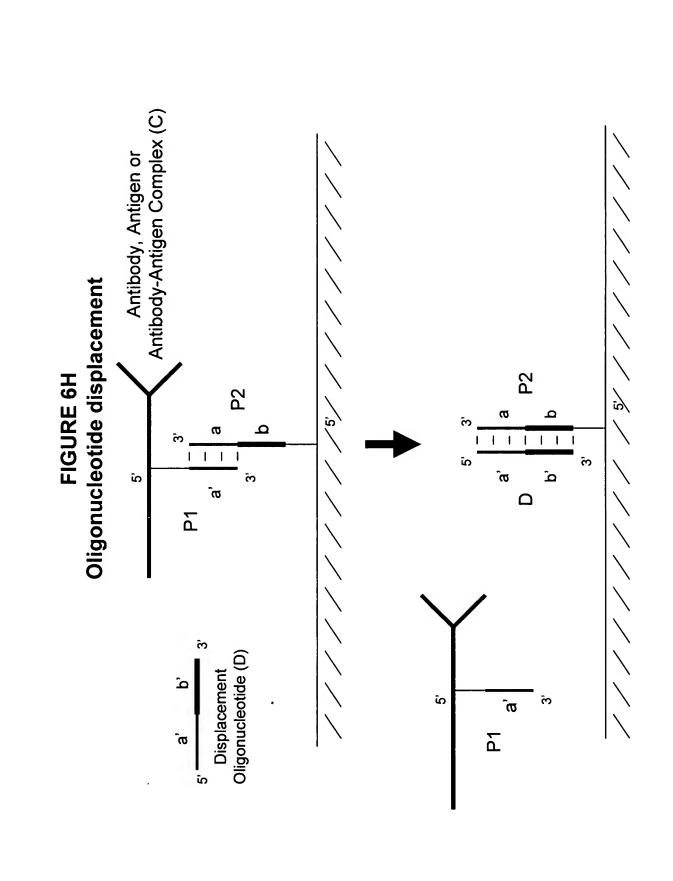
Polymerase and restriction endonuclease-mediated release FIGURE 6E











... □ **P**2 , 0 Ø က က ດົ ີ້ຕ 7 Strand Displacing Polymerase Oligonucleotide extension Hybridization P2 Antibody, Antigen or Antibody-Antigen Complex (C) Ø ດ່ I 1 က ڡٛ ື້ P2 ത I = I1 -<u>ښ</u> ຸດ **ົ** ຕ 7 ດ໌ ີ້ຫ 7 Displacement Oligonucleotide (D) ŝ ۵,

**FIGURE 6I** 

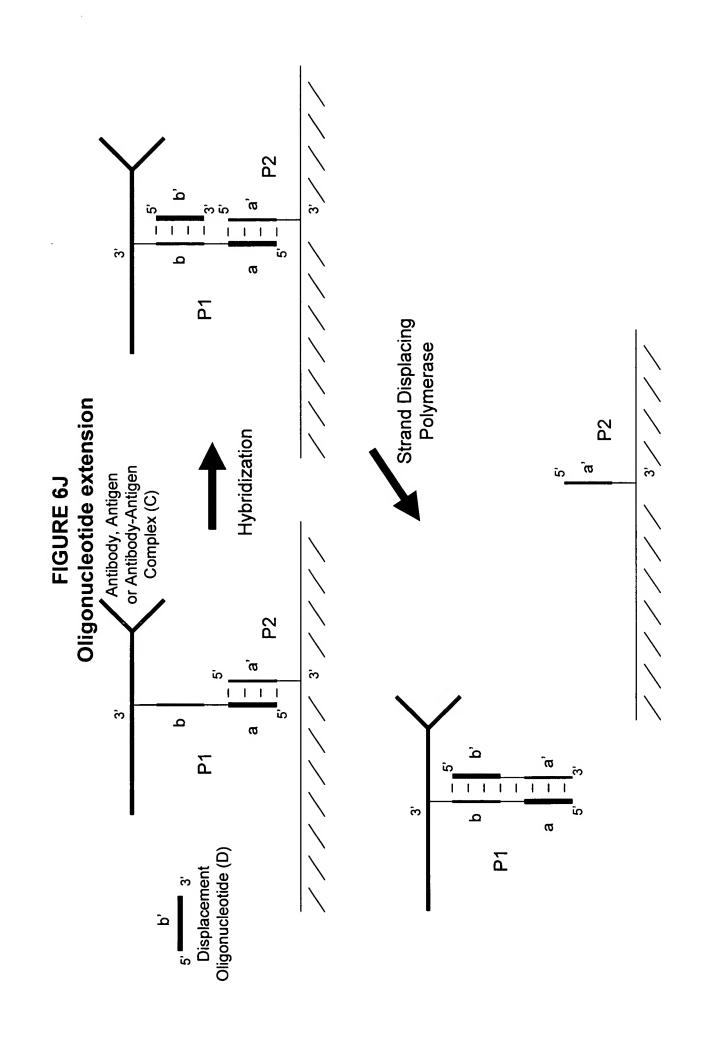
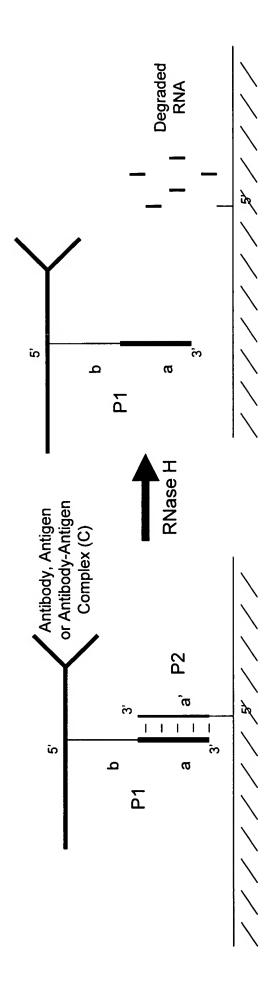


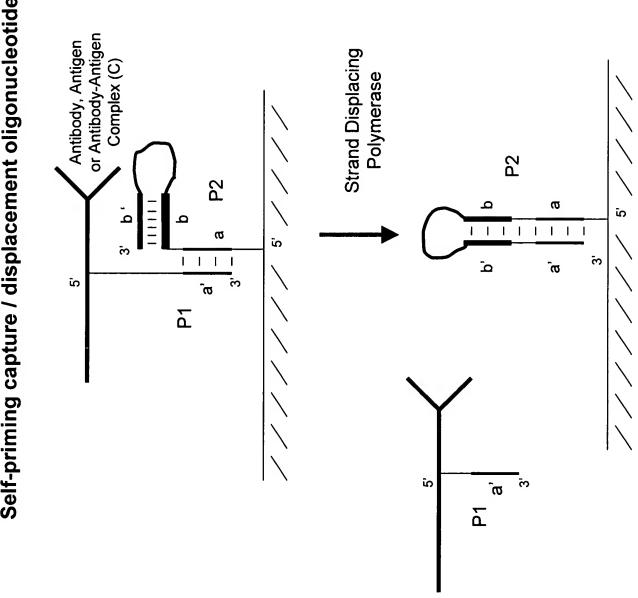
FIGURE 6K RNase H release

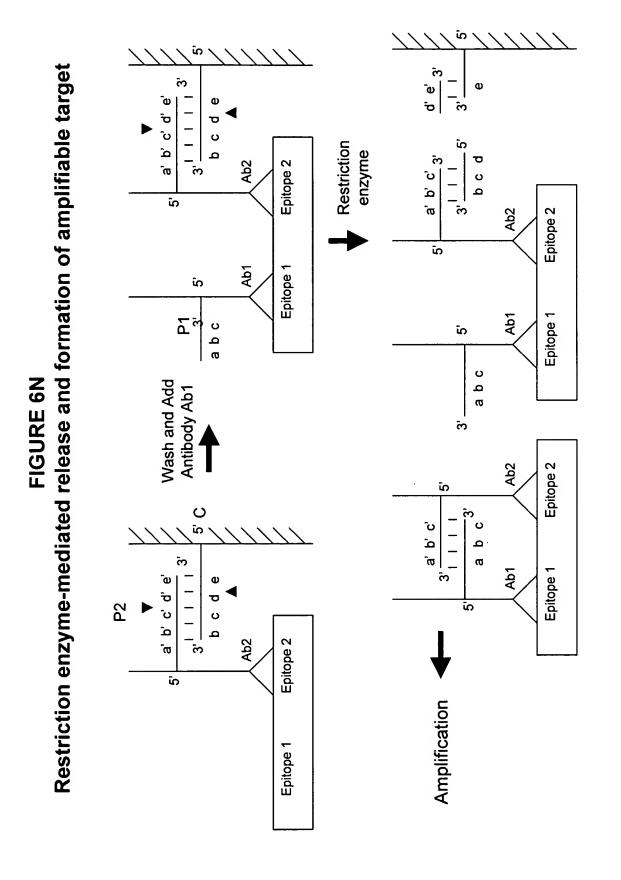


P2 ັດ Ω Degraded RNA Φ RNase H Antibody, Antigen or Antibody-Antigen Complex (C) **P**2 'n 1 1 1 1 ົດ Ø ρ 7

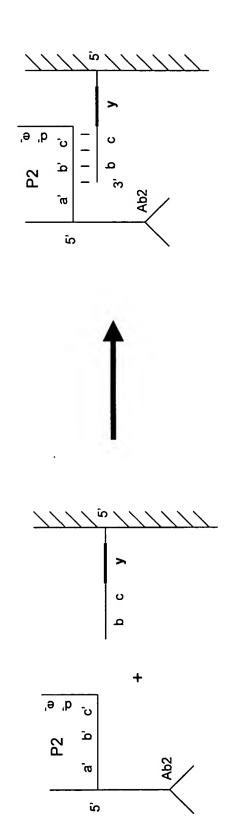
FIGURE 6L RNase H release

Self-priming capture / displacement oligonucleotide FIGURE 6M

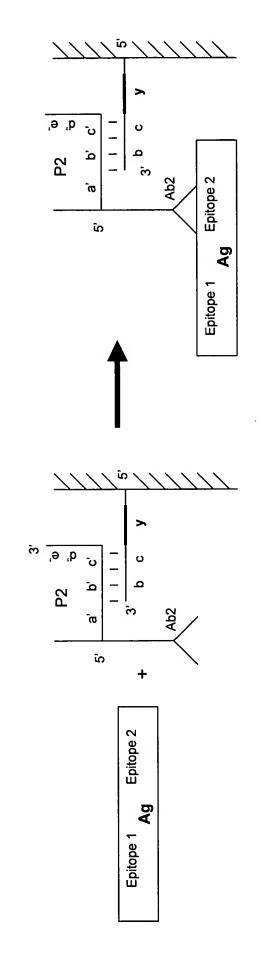




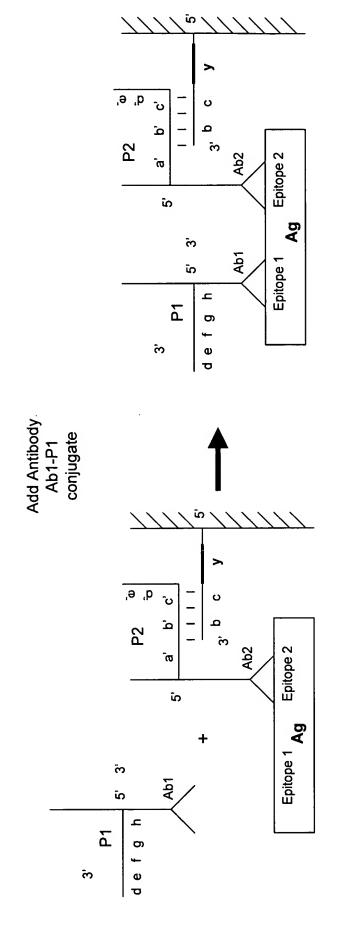
Immobilization of antibody-probe conjugate by hybridization of a probe oligonucleotide to a capture oligonucleotide FIGURE 7A



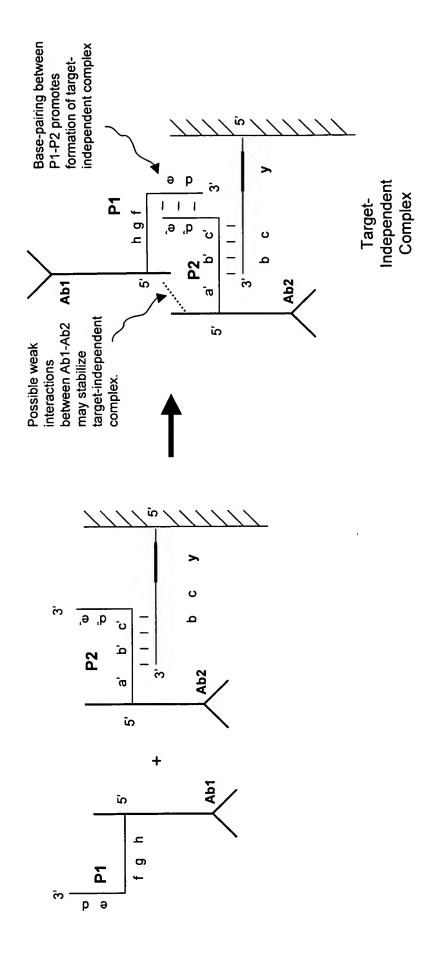
Binding of target ligand to antibody-probe conjugate immobilized by a capture oligonucleotide **FIGURE 7B** 



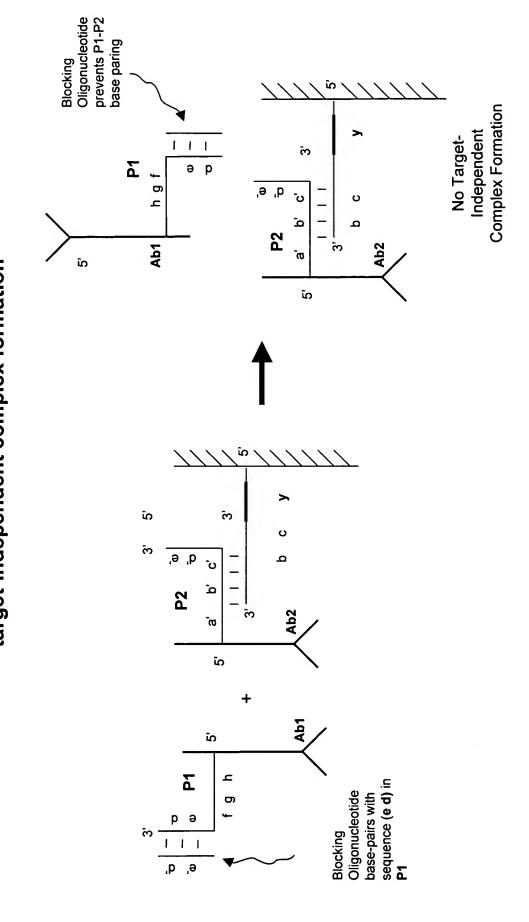
Formation of immobilized two-site "sandwich" complex by binding second antibody-probe conjugate to target ligand **FIGURE 7C** 



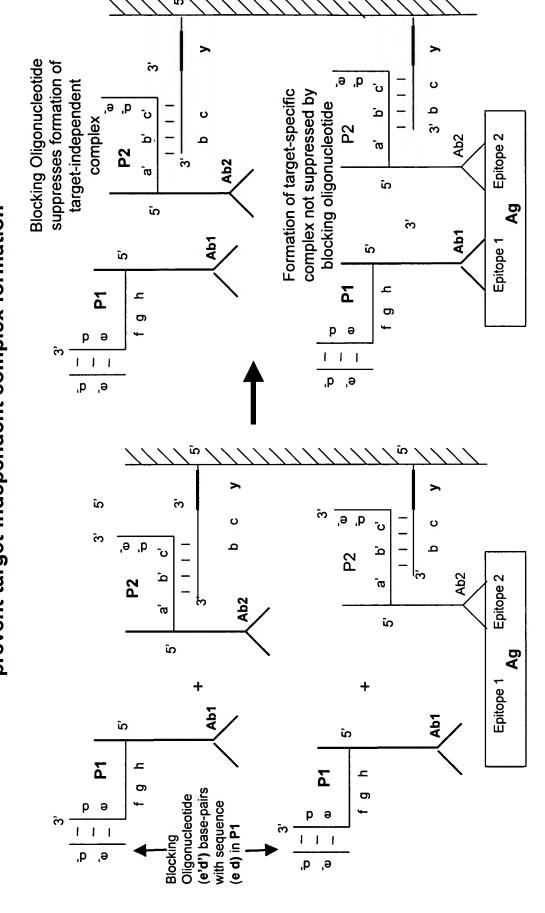
Formation of target-independent complex involving probe-probe (P1-P2) interactions **FIGURE 7D** 



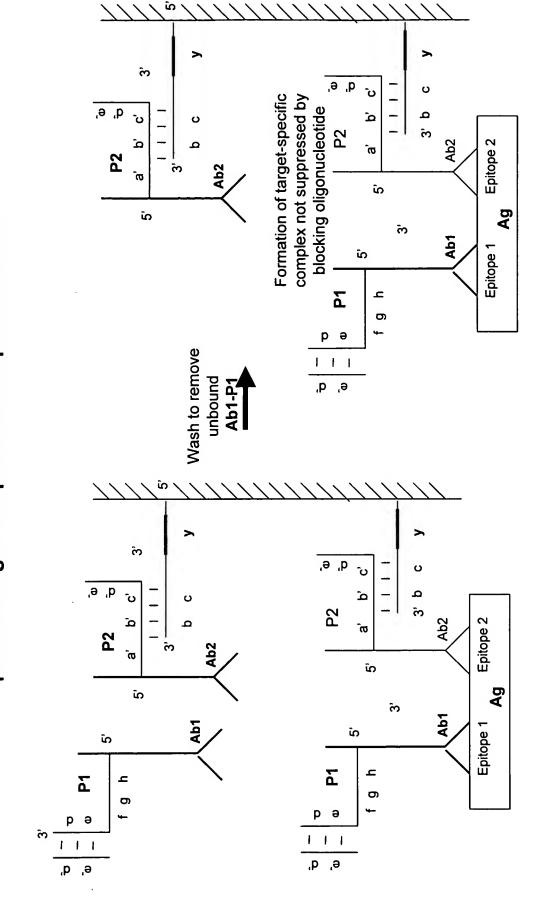
Use of blocking oligonucleotide to suppress P1-P2 interactions leading to target-independent complex formation **FIGURE 7E** 



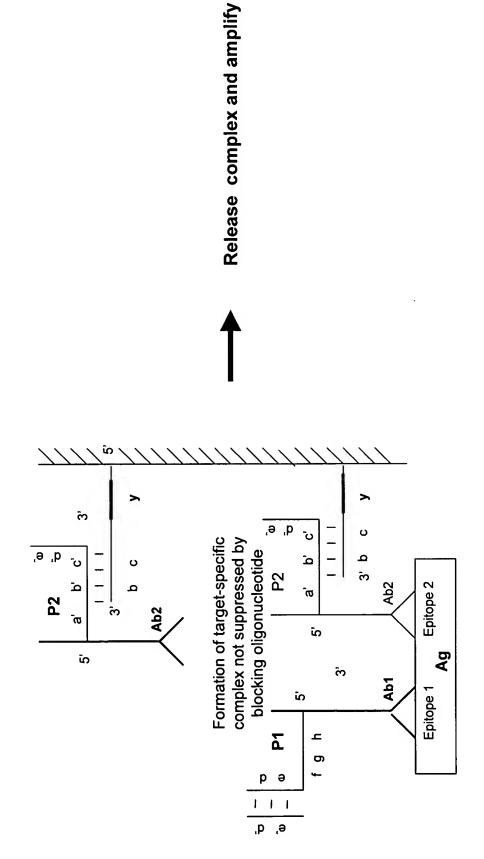
Use of blocking oligonucleotide to suppress P1-P2 interactions and prevent target-independent complex formation **FIGURE 7F** 

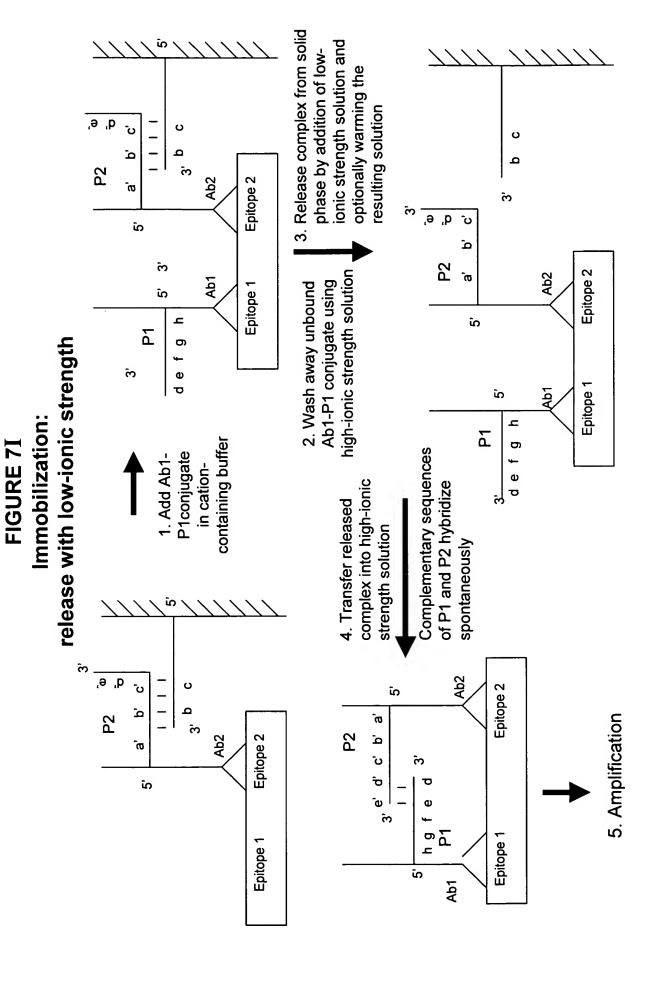


Use of blocking oligonucleotide to suppress P1-P2 interactions and prevent target-independent complex formation FIGURE 7G

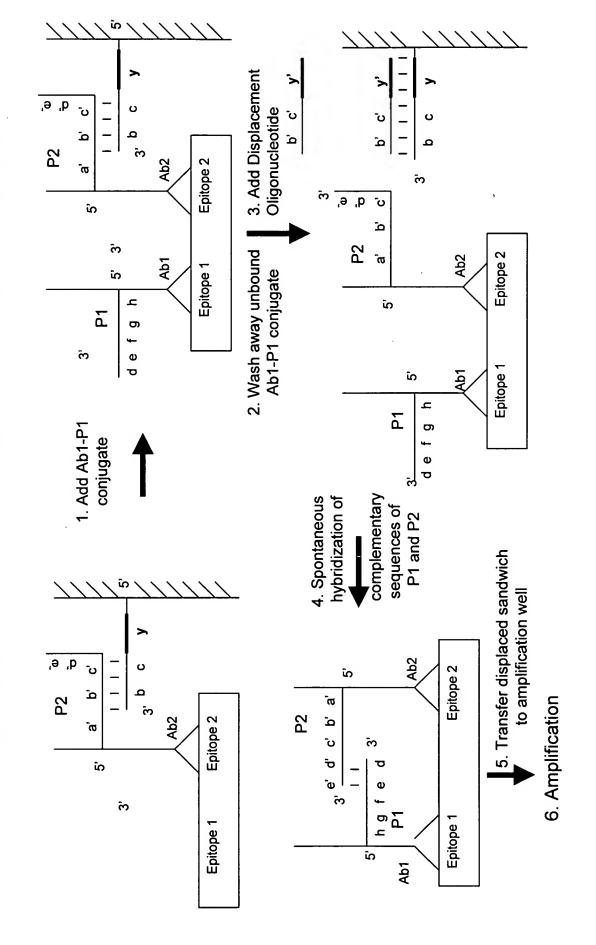


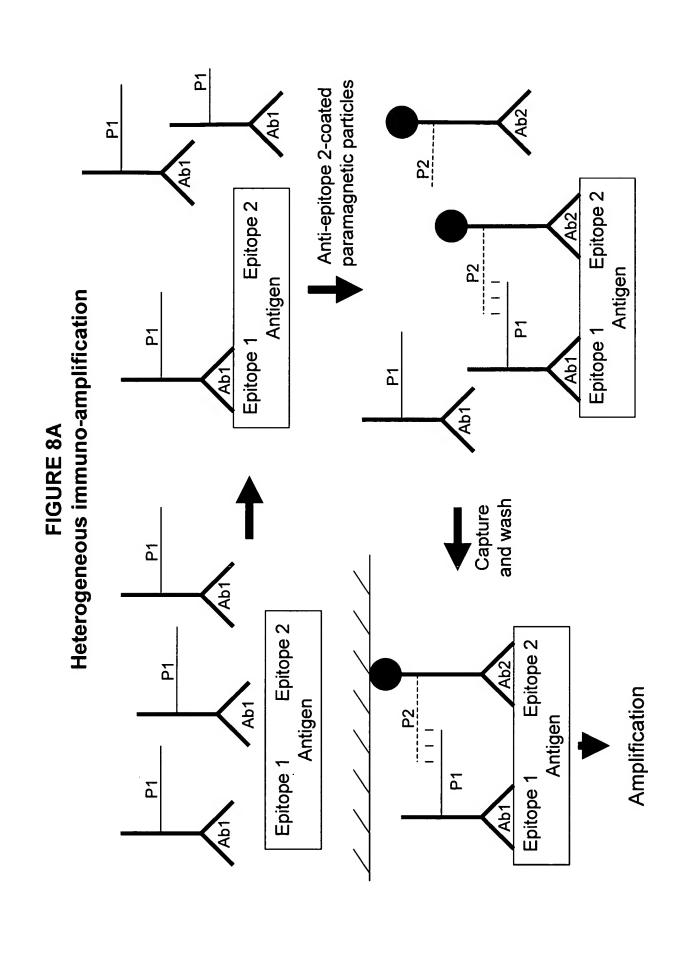
Use of blocking oligonucleotide to suppress P1-P2 interactions and prevent target-independent complex formation **FIGURE 7H** 

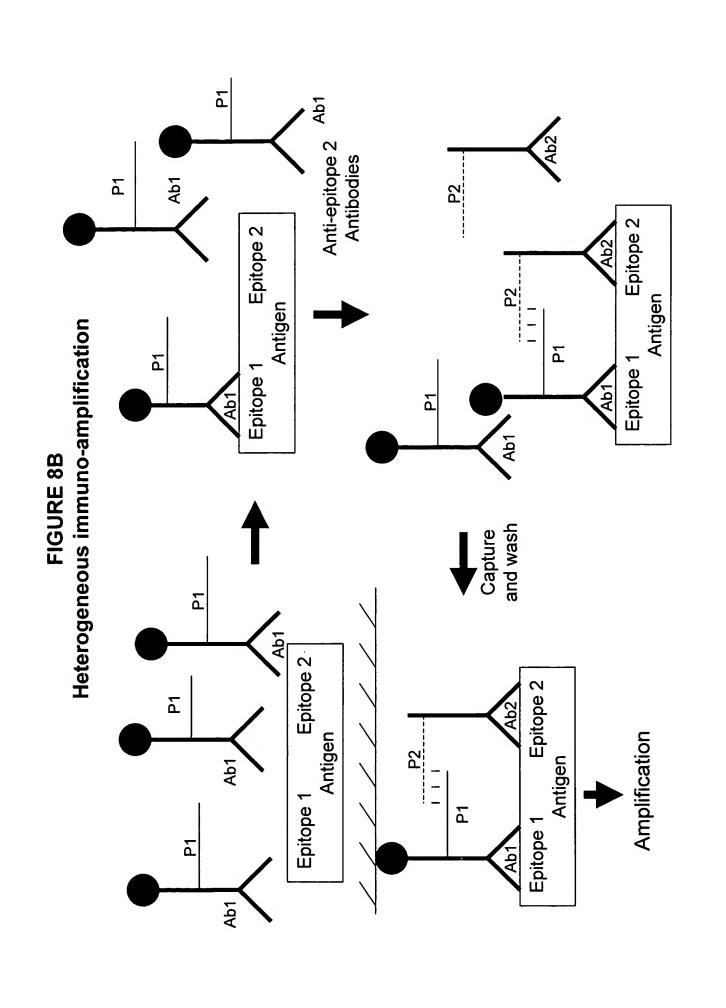




Heterogeneous formation and displacement of amplifiable complex **FIGURE 7J** 



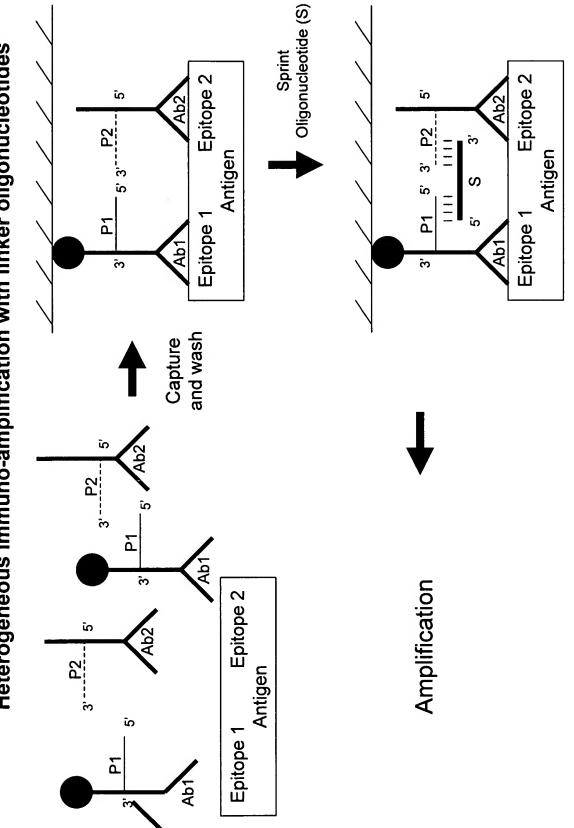




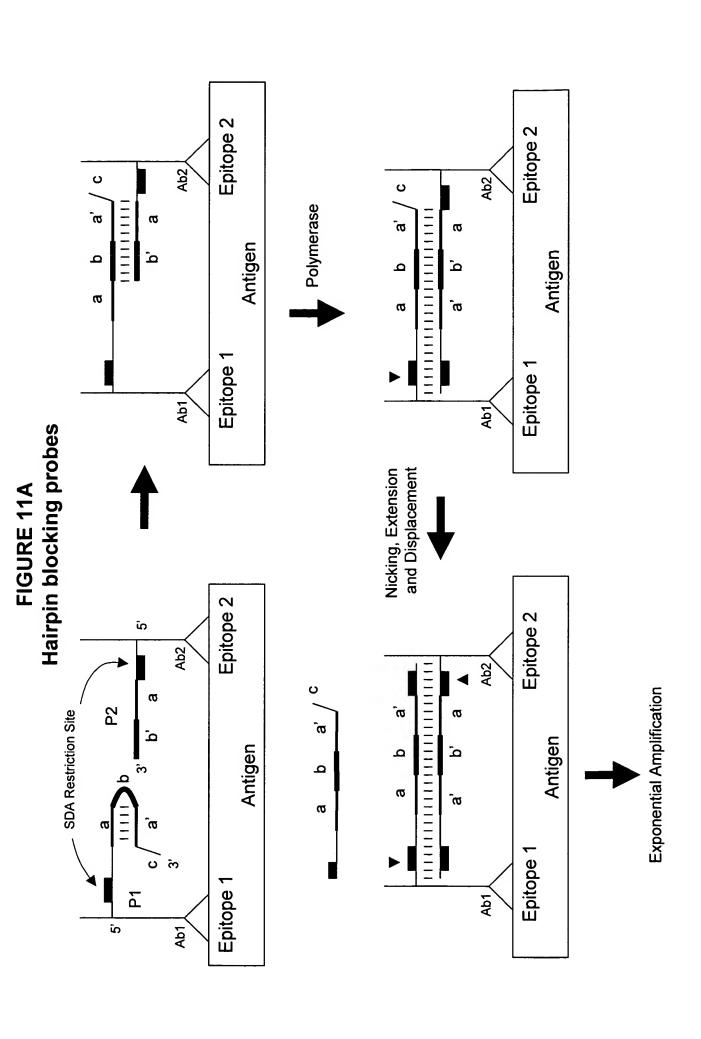
7 P2 7 FIGURE 8C: Heterogeneous immuno-amplification Ab1 Epitope 2 7 P2 Antigen 7 Epitope 1 Epitope 2 P2 Antigen 7 Capture and wash Epitope 1 P2 Epitope 2 7 Amplification Ab1 P2 Antigen 7 Ab2 Epitope 1 **P**2

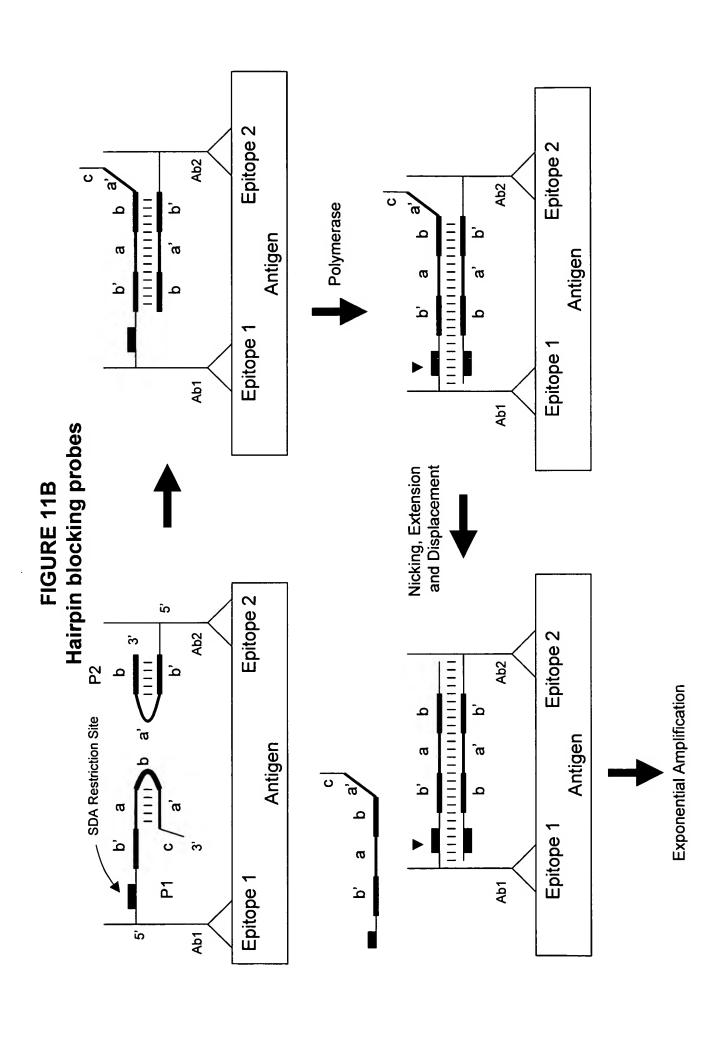
 ▼ Cleave Scissile
 ■ Cleave Scis Linkage Epitope 2 Epitope 2 Heterogeneous immuno-amplification with scissile linkage P2 Antigen Antigen 7 Epitope 1 Ab1 7 Epitope 1 Ab1 remove unbound Ab1 Wash to Transfer liquid phase **FIGURE 8D** <u>6</u> 7 Epitope 2 Amplification P2 Epitope 2 Antigen 7 P2 Epitope 1 Antigen Ab1 7 Epitope 1 Ab1

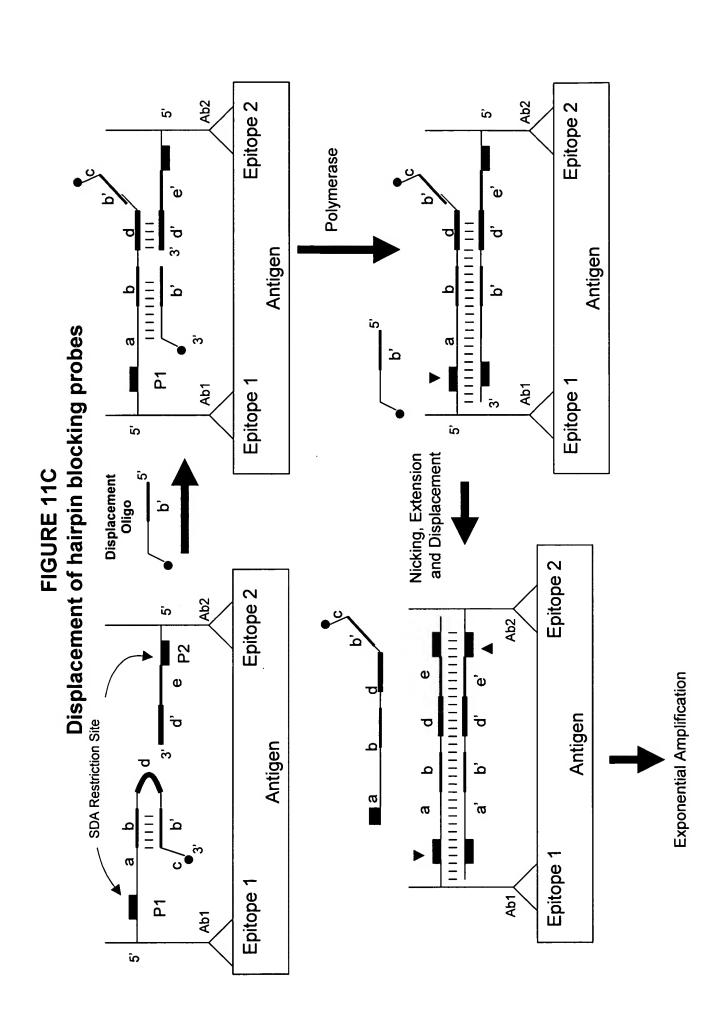
Heterogeneous immuno-amplification with linker oligonucleotides FIGURE 9

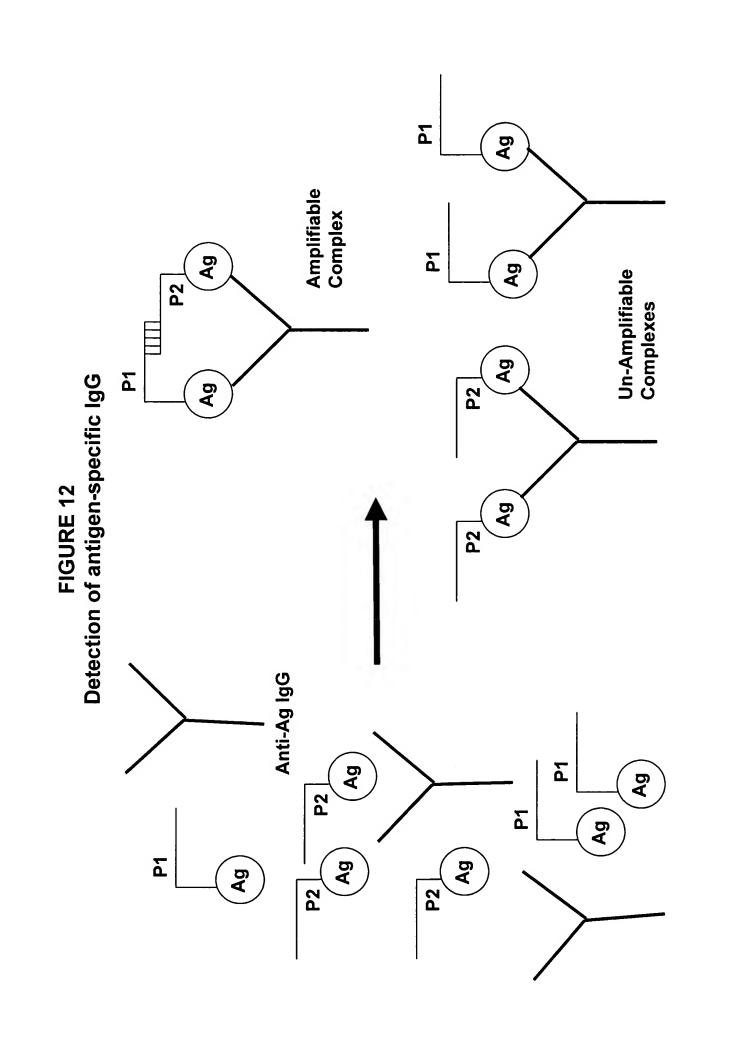


Sec 2 Polymerase Epitope 2 Epitope 2 P2 Antigen Antigen = Universal immuno-amplification system Epitope 1 Epitope 1 7 Sec1 FIGURE 10 Add Universal Oligonucleotidelabeled anti-F<sub>c</sub> Antibodies Amplification Epitope 2 Antigen Epitope 1

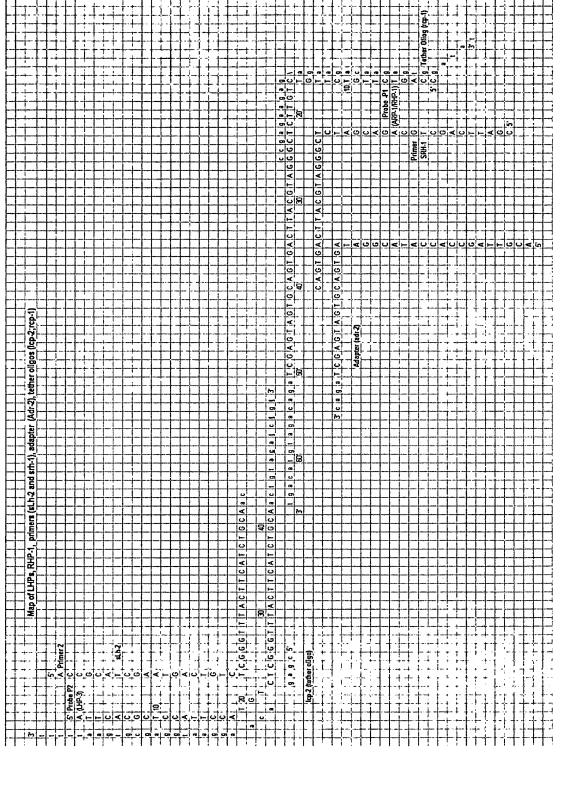




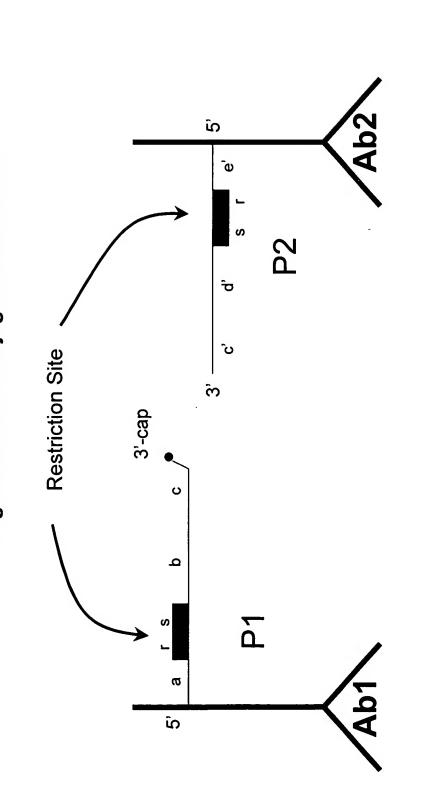




Map of probes, primers, tether oligos for binary immuno-SDA FIGURE 13



Immuno-SDA using capped oligonucleotide probes: Mixing of antigens and oligonucleotide-conjugated antibodies **FIGURE 14A** 



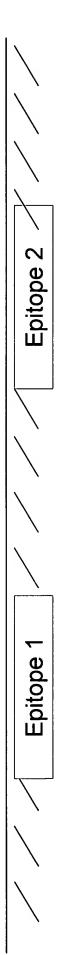
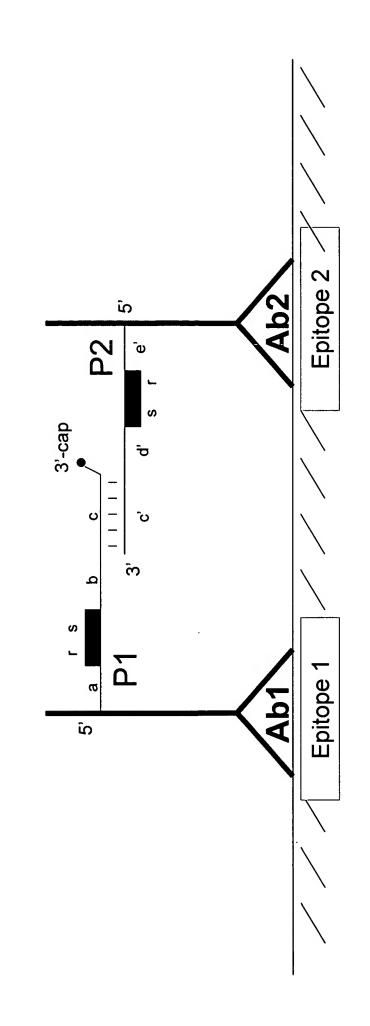
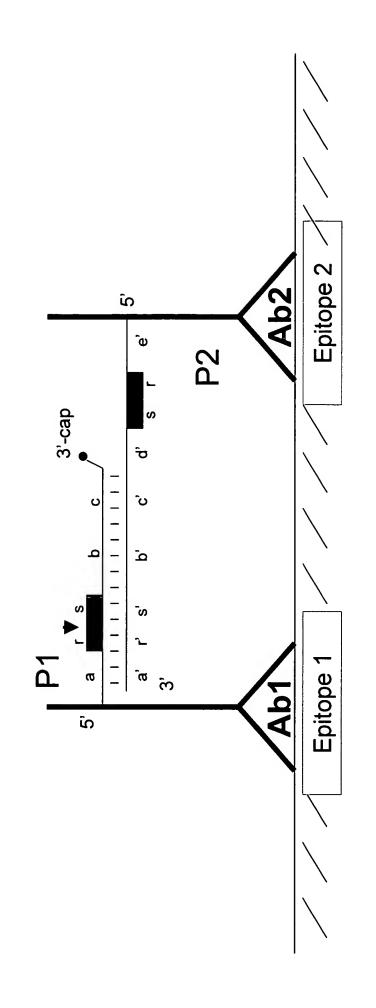


FIGURE 14B
Hybridization of adjacent probes



Polymerase extension and restriction enzyme nicking **FIGURE 14C** 



Extension from nick and displacement of 3'-capped fragment **FIGURE 14D** 

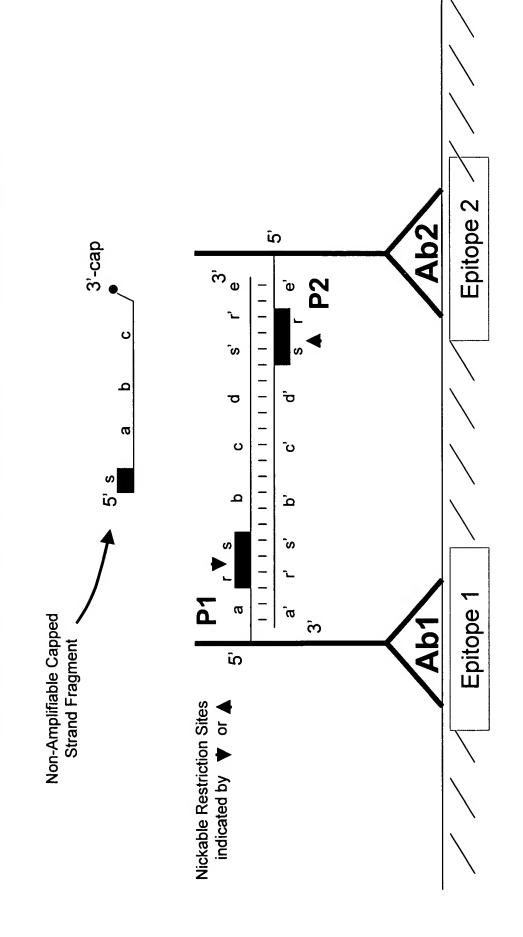
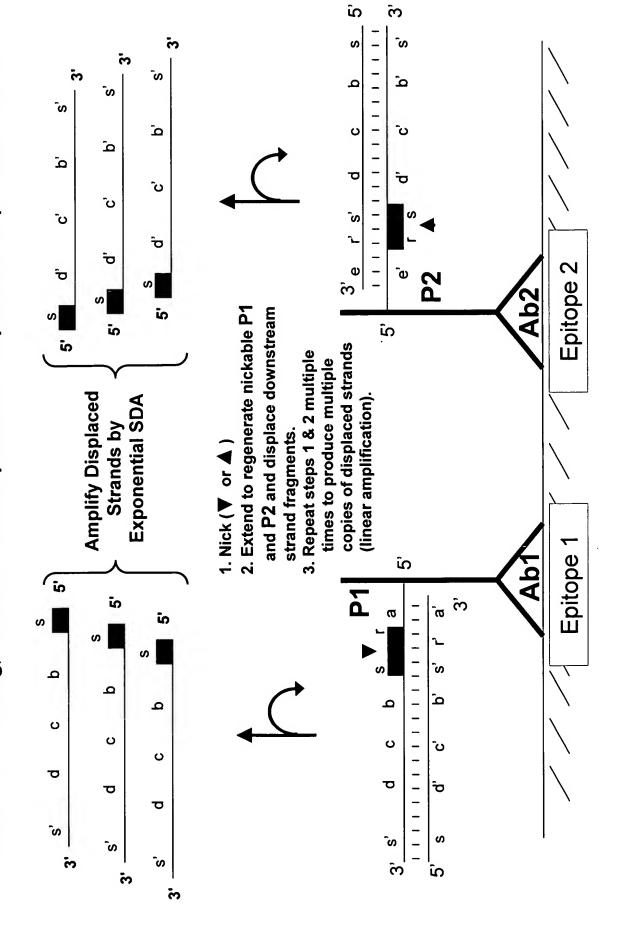


FIGURE 14E: Nicking, extension and displacement to produce amplifiable strands



Two-color, real-time fluorescence profile for immuno-SDA detection of IL-8 **FIGURE 15A** 

